REMARKS

Claims 1, 17 and 18 which were withdrawn from consideration in this application were canceled. The subject matter of these claims (which was considered to represent a separate invention) is the subject of the claims in U.S. 6,692,941 issued 17 February 2004, on the parent application of which this is a divisional.

Claims 19-22 are pending.

Claims 19-22 are rejected under 35 U.S.C. § 102 (b), or in the alternative, under 35 U.S.C. § 103(a) as allegedly obvious over Daniels, *et al.* (U.S. Patent No. 3,265,579). This is the only basis for rejection outstanding. Daniels discloses bovine growth hormone ("bGH") which is purified from pituitaries. The Office action asserts that the bGH disclosed in Daniels appears to be substantially the same as that of the instant claims. The Board of Patent Appeals and Interferences agreed, reasoning that (1) no objective evidence of record supported Applicants' position that recombinant bGH (r-bGH) and pituitary-derived bGH (p-bGH) are distinct proteins and (2) the methods of Daniels encompass 100% pure bGH preparations, and thus can presumably be free of the causative agent of bovine spongiform encephalopathy (BSE), *i.e.*, Mad Cow Disease.

Applicants believe the inherent differences, generally known in the art, between recombinantly produced bGH and bGH extracted from pituitaries (p-bGH) have been overlooked and that the most significant difference – a guarantee of freedom from the causative agent of BSE has been improperly ignored. Applicants believe that the evidence of commercial success of the recombinantly produced product is strong evidence of this difference, and testimony is submitted herewith to verify that this is the case.

Art-Known Structural Differences

As a preliminary matter, it is straightforward that recombinant bGH ("r-bGH") is not substantially the same as pituitary purified bGH ("p-bGH"), and therefore Daniels disclosure of p-bGH does not anticipate the r-bGH of the instant claims. As noted in paragraph 7 of the Declaration of Dr. Petersen, in order to obtain reasonable amounts of bGH, pools of >1000 pituitary glands would have to be employed. For that reason, bGH isolated from pituitary is a mixture of four structurally distinct species of bGH produced *in vivo*. *See* Secchi, *et al.*, *J. Chromatogr. B. Biomed. Sci. Appl.* (1997) 688:161-177. An allelic polymorphism at amino acid position 127 of the bGH gene results in a pair of structurally distinct species of bGH being produced *in vivo*. The two alleles differ in a single amino acid at position 127, where the either leucine ("L" form) or valine ("V" form) is found. When both alleles are present, these two forms are produced at a 1:2 ratio of the V to the L form and can be distinguished by reversed-phase high pressure liquid chromatography (HPLC). *See, e.g.,* Exhibit A at Figure 1.

In addition, bGH is originally synthesized as a larger 217 amino acid precursor protein with a 26 amino acid long signal sequence peptide, which is removed via cleavage. The cleavage site for the bGH signal sequence peptide is ambiguous, and thus it sometimes is cut imprecisely, thereby giving rise to two distinct forms of the bGH protein. The precise removal of the 26 amino acid signal sequence peptide results in a 191 amino acid long bGH starting with the amino acid alanine (the "A" form). An imprecise removal cleaves away the first alanine as well as the 26 amino acid signal sequence peptide, resulting in the smaller sized bGH that is 190 amino acids long that starts with the amino acid phenylalanine (the "F" form). The "F" and the "A" forms are produced in

equimolar quantities and are distinguishable on the basis of their charge differences. *See* Exhibit A at Figure 3.

As a result of these differential cleavage products and allelic polymorphisms, four species of bGH are naturally synthesized in the pituitary, as illustrated below:

	Amino acid position		**Designation**
	127		
Amino terminal A-F----------	L	---------- Carboxyl terminal	**AL**
Amino terminal A-F----------	V	---------- Carboxyl terminal	**AV**
Amino terminal F----------	L	---------- Carboxyl terminal	**FL**
Amino terminal F----------	V	---------- Carboxyl terminal	**FV**

Therefore, bGH isolated from pituitaries will contain these four different forms of bGH, each with a distinct amino acid sequence, irrespective of the method of purification.

The recombinant bGH, claimed (r-bGH), is made as a single form of bGH. The source of r-bGH is a single gene expressed in a host cell. Thus, r-bGH will always be either a pure "L" species or a pure "V" species, depending on which allelic species is used for the transformation. In most host cells, such as bacterial cells, r-bGH is the "A" form because the ambiguity of signal peptide cleavage site is not recognized. Therefore, a bacterial host transformed with a DNA encoding the "AL" species produces only the "AL" species and none of the other three species. The homogeneity of r-bGH produced in bacterial cells is easily and unmistakably discernible when compared with pituitary derived hormone (p-bGH). *See, e.g.*, Exhibit A at Figure 3 (showing a single band for r-bGH and multiple bands for p-bGH). While some host cells, such as murine fibroblasts, can result

in both the "A" form or the "F" form, r-bGH from such a source still has no more than two species of bGH, and thus is still distinct from that of the four species found in p-bGH. *See, e.g.*, Exhibit B.

p-bGH is also structurally distinct from r-bGH in the chemical modifications present; p-bGH is extensively modified, while r-bGH is not. *See* Exhibit A at 14744. The amino acid aspartate at position 128 is derivatized to "β-linked" isoaspartate in 20% of bGH isolated from bovine pituitary. No such modification is observed in r-bGH preparations. *Id.* These modifications appear to be a result from *in vivo* modification in the pituitary rather than the extraction procedure. *See* Exhibit A at 14747 (stating that such derivitization should not occur at the conditions used to purify the hormone and suggesting that such derivatized bGH "may be a component of somatotropin *in vivo*"). While the exact significance of this derivitization is unclear, *in vitro* aging of human growth hormone results in the same derivitizations. *See* Exhibit C.

Furthermore, p-bGH is also deamidated and partially deleted at its amino terminus while r-bGH is not. Before 1985, the art-known procedures for making p-bGH, including the Li and Wilhemi methods taught in Daniels, employed steps that favored the formation of modified protein forms. *See* Exhibit D at 8522 (stating that most existing procedures employ some steps which favor the formation of modified protein forms). The modifications were caused by proteolysis, deamidation, oxidation, or partial denaturation, which occurred as a result of lengthy purification procedures in relatively harsh conditions. *Id.* Indeed, the p-bGH lots distributed by the National Institute of Health as bGH standards contained half as many amino-terminal deleted versions as the full-length version of the bGH. *See* Exhibit A at 14743. r-bGH, on the other hand, does not require such lengthy purification procedures, and thus undergoes none of these modifications. *See* Exhibit

D at Figure 1 (showing deamidated forms of bGH in p-bGH made by the Li method as taught in Daniels).

As the enclosed exhibits show, there are inevitable structural differences that distinguish recombinantly produced bGH from that isolated from pituitaries as in the method of Daniels.

Applicants concede that the foregoing evidence could have been adduced during prosecution, but the significance of these differences appeared less important than the clear advantage conferred by the ability to guarantee that recombinantly produced bGH is free of the causative agent for BSE or more generically, transmissible spongiform encephalopathy (TSE), associated with not only bovine brains, but also many other animal species as well as humans.

The Difference that Really Matters

To emphasize this point, submitted herewith are declarations from Raymond Bradley and Robert B. Petersen, experts in this field, who conclude that pituitary-derived bGH carries significant risks of transmitting TSE to cattle, and hence to humans.

Dr. Bradley provides, in paragraphs 1-14 of his declaration, background information based on his expertise on the nature of various TSE's. In view of the manner in which pituitaries are obtained from bovine sources, and in view of the inability to ascertain the possible presence of the infectious agents in slaughtered cattle, as set forth in paragraphs 15-22, Dr. Bradley concludes, for the reasons summarized in paragraph 23, that, as stated in paragraph 24, where bGH is derived from pituitaries there is a risk of BSE contamination in the hormone preparation. Further, as stated in paragraph 25 the use of bGH in cattle, if that bGH were derived from pituitaries, would permit transmission of BSE in the cattle population.

As set forth in paragraphs 26-31, where the manner of administration and consequences thereof are set forth, it is clear that transmission of TSE would be "more than likely" if the pituitary extracted BSE were contaminated with a TSE agent. Dr. Bradley contrasts this with recombinant bGH which carries no risk of TSE infectivity.

Dr. Bradley's opinion is summarized in paragraphs 34-36 that "*there is sufficient scientific justification for regulatory agents to prohibit the use of pituitary-derived bGH in cattle.*" Thus, in practical terms, recombinant bGH differs from pituitary bGH in that the recombinant form is useful wherein pituitary-derived bGH is not. The reason for the lack of utility resides in an actual physical difference in the preparations.

Dr. Bradley's testimony is complemented by that of Dr. Petersen who further notes that it is difficult, if not impossible, to test for the presence of BSE in cattle on a routine basis (see, in particular, para. 11) and notes the economic consequences of the spread of this condition. As noted above, TSE's are cross-infective among various animals and thus in addition to the risk of BSE *per se*, the human counterpart, or Creutzfeldt-Jakob disease (CJD), is also a risk factor were pituitary-derived bGH to be used in cattle. As stated in paragraphs 18-23, the bGH derived from pituitary carries significant risk of a TSE infective agent, and as confirmed in paragraphs 24-29, recombinant bGH does not. Again, this difference arises from physical differences in the actual material that is represented by "pituitary-derived bGH" and "recombinantly produced bGH."

Commercial Success

These significant differences are directly responsible for the commercial success of recombinant bGH marketed by Monsanto under the trademark Posilac™. As evidence of this success, submitted herewith is the declaration of an economic expert, Dr. Charles Mahla.

Dr. Mahla's conclusions are summarized in paragraph 4 of his Declaration and their basis is outlined in paragraphs 5-8. Dr. Mahla concludes that over a nine year period, Posilac™ has generated more than $1.6 billion in revenues, leading Monsanto to claim that Posilac™ "has become the largest selling dairy animal health product in the United States."

The facts in Dr. Mahla's declaration, combined with the testimony of Dr. Petersen and Dr. Bradley confirm that this commercial success has been possible only because the bGH is produced recombinantly. Clearly the use of pituitary-derived bGH, even if it could be produced in sufficient amounts in a cost-effective manner, would be unthinkable due to the risk of spreading TSE to both cattle and humans.

The significance of such assurance of lack of risk has also been recognized by the U.S. PTO. For example, enclosed herewith is U.S. 5,618,789 which issued with claim 1 directed to

> A composition comprising a recombinant functional human factor VIII free of viral contaminants that affect humans and a pharmaceutically acceptable carrier.

Pharmaceutical compositions of factor VIII isolated from blood were well known in the art prior to the date of application for this patent, as described in the patent itself beginning in column 2, line 43 through column 3, line 10. The prosecution history reveals that the distinction drawn between the claimed composition and that of the prior art was the assurance that viral contamination (such as HIV) would not be present in the recombinantly produced material. Similarly, as extensively shown in the present case, it is only recombinant bGH, not pituitary bGH that can be assured as free of TSE-causing contaminants.

In summary, both the commercial market and the PTO recognize the importance of assurance of freedom from contaminants and side effects.

The uniformity of preparation assured by recombinant production of bGH also contributes to its commercial success as it will exert its functions reproducibly and does not produce some of the side effects that are exhibited by pituitary-derived hormone. These characteristics make the use of r-bGH on a widespread basis attractive to dairy farmers.

p-bGH is functionally heterogeneous because different preparations of p-bGH elicit different combinations and levels of the various biological activities. For example, one preparation of p-bGH resulted in increased levels of glucose and insulin (diabetogenic effect) in lactating cows, while another lacked a diabetogenic effect. *See* Exhibit E. Amino-terminal deletions in a p-bGH preparation were implicated as the cause of decreased milk yield in cows. *See, e.g.,* Exhibit E at 50 (concluding that the "presence of amino-terminal deletions in p-bGH preparations was implicated as an important cause for lowered milk response").

r-bGH lacks the side effects elicited by p-bGH. For example, r-bGH lacks lipolytic activity, *i.e.*, catabolizing fat, *in vitro* (*See* Exhibit F) while p-bGH stimulates significant catabolism of fat in an *in vitro* assay. *Id.* The diabetogenic ability of r-bGH is significantly less than that of p-bGH as r-bGH has less ability to inhibit insulin-stimulated fall in blood glucose levels when compared with p-bGH. *See, e.g.*, Exhibit E at 1984. It has been suggested that the diabetogenic activity may be caused by a fragment of bGH present in the pituitary preparation. *See, e.g.*, Exhibit G at 580.

r-bGH also has unexpectedly greater efficacy in enhancing milk production. While both r-bGH and p-bGH result in increased milk yield in cows, r-bGH has greater potency. *See, e.g.,* Exhibit H. In one study, cows receiving r-bGH increased their milk yield from 23 to 41%, while the same dose of p-bGH resulted in only a 16.5% increase in milk yield.

Another commercial advantage is that the particular species of recombinant hormone can be chosen to optimize results. For example, the "AV" species of r-bGH elicits a greater milk yield than the "AL" species when administered to cows. *See* Exhibit F at 50. By specifically generating only the "AV" species by recombinant means, milk production can be maximized.

The Daniels Protein

The risk of contaminants and side effects is particularly high in the cited Daniels protein. Daniels fails to teach or suggest the conditions necessary to generate 100% pure p-bGH, and therefore such preparations are functionally heterogeneous and carry significant risk of BSE contamination.

Daniels discloses the use of the methods such as those of Li and Wilheimi for the initial extraction step. *See* Daniels at column 1, lines 39-44. Li and Wilheimi methods both employ high pH conditions, *i.e.*, pH of 10.5 and 11, respectively, using $Ca(OH)_2$, for the first extraction step. *See* Exhibit D at 8521. As discussed above, such harsh conditions result in bGH chemical modification, contributing to the functional heterogeneity of the p-bGH species.

Daniels' method starts with crude bGH prepared by Wilhelini, *J. Biol. Chem.* (1948) 176:737 followed by a chromatography step, using gel exclusion chromatography. This results in a single peak. However, gel exclusion chromatography separates components in a solution by size. Small molecules are slowed or trapped by the pores in the gel beads filling the column, while large molecules, too large to fit into the pores, slide past the beads and get to the bottom of the column first. Thus, a single peak on a chromatogram merely indicates that all of the detectable components of that fraction are within the same size range, but provides no indication of the actual purity of the examined fraction. Moreover, it was recognized in the art that prolactin was a major hormonal

contaminant of bGH, often representing 1-5% of the salt-extracted protein from a given pituitary preparation. *See, e.g.*, Exhibit G at 579 (stating that p-bGH preparations obtained by salt extraction contained 1-5% prolactin). Gel exclusion chromatography would not separate the biologically active form of prolactin as its molecular weight of 23kD is only one 1kD larger than that of bGH with a weight of 22kD.

Daniels also would not be seen as disclosing pure hormone by the art-skilled practitioners because it is clear that before publication of Bell's method in 1985 (Exhibit D), the available p-bGH preparations were impure. This is seen by the fact that researchers failed in their attempts to crystallize bGH - a process that requires a relatively pure protein preparation. While p-bGH preparations had been available for almost four decades before 1985, numerous efforts by several crystallographic groups failed to successfully crystallize bGH. Those of skill in the art attributed this failure to impurities in the p-bGH preparations. *See* Exhibit D at 8520 ("Re-examination of samples from some of these preparations ... convinced us that previous failures in crystallization attempts may have arisen due to inadequate purity of the preparation.").

Even Bell succeeded in producing only about 95% pure p-bGH by using a combination of a mild extraction procedure, followed by two rounds of ion-exchange chromatography. More specifically, the Bell method included both extraction and DEAE-cellulose ion-exchange chromatography ("DEAE") steps to result in 80% pure p-bGH. Addition of a carboxymethyl-cellulose ion-exchange chromatography ("CM") step generated p-bGH of more than 95% purity. Because Daniels method relied only on size exclusion chromatography, it is highly unlikely that such method would have resulted in a significantly pure preparation of p-bGH, much less a 100% pure preparation.

In summary, although Daniels' bGH might appear as a single peak in gel exclusion chromatography, this is not conclusive as to its purity and taken in context of the knowledge of the art, Daniels' preparations contain significant impurities. The nature of these impurities would inherently be very different from those found in associated with recombinantly produced bGH regardless of the level of purification of that protein. The most significant possible impurity, of course, would be the causative agent for BSE.

CONCLUSION

Recombinantly produced bGH has been an unqualified commercial success in the United States, generating $1.6 billion in sales over nine years. The commercial success of recombinant bGH has been documented by the Declaration of Dr. Mahla and the exhibits attached thereto. This commercial success is directly attributable to the fact that it is recombinantly produced bGH because the hormone prepared by this process inherently has favorable physical characteristics that distinguish it from any pituitary-derived bGH preparation. The most significant of these characteristics is its guaranteed freedom from the causative agent of BSE, an assurance that can never be made with respect to bGH from pituitaries, as verified by the declarations of Drs. Bradley and Petersen. In the event that such a guarantee, despite its tremendous societal and economic significance does not provide what the Office considers sufficient assurance that differences exist, it is also demonstrated above that pituitary-derived bGH is inherently heterogeneous regardless of the actual presence or absence of BSE causing agents both structurally and functionally. The particular preparation of bGH cited by the Office, that of Daniels, is clearly physically different from the recombinantly produced materials due to the crudity of the preparation steps and the inconclusiveness of the criterion for assessing level of purity. For these reasons, the recombinant

form of bovine growth hormone claimed in the present application is neither anticipated nor rendered obvious by the Daniels disclosure. Accordingly, passage of claims 19-22 to issue is respectfully requested.

If a telephone conference would be helpful to resolve any issues, a telephone call to the undersigned would be both invited and appreciated.

In the event the U.S. Patent and Trademark office determines that an extension and/or other relief is required, applicant petitions for any required relief including extensions of time and authorizes the Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket no. 220002016004. However, the Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

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Respectfully submitted,

By Kate H. Murashige

Kate H. Murashige
Registration No.: 29,959
MORRISON & FOERSTER LLP
3811 Valley Centre Drive, Suite 500
San Diego, California 92130
(858) 720-5112